ORIGINAL RESEARCH

An update of the invasion status of *Rapana venosa* (Mollusca: Gastropoda) in the Río de la Plata estuary

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**ABSTRACT.** *Rapana venosa*, a gastropod of Asian origin, has become a highly successful invasive species in various coastal-estuarine ecosystems worldwide. It was first recorded in the Río de la Plata (RdIP) in 1999 and has since expanded its range along the Argentine and Uruguayan Atlantic coast, and recently in southern Brazil. This study collected *R. venosa* samples during spring 2017 in the RdIP (middle/outer) and on the Uruguayan coast of the Argentine-Uruguayan Common Fishing Zone. The study found that 16% of stations surveyed contained *R. venosa* specimens with a density of 3.88 kg m⁻². Among the 119 specimens analyzed, males predominated, and imposex was observed in two organisms. Most individuals had a high epibiont coverage, and 10% were found consuming native bivalves. Isotopic analysis ($\delta^{15}N$ and $\delta^{13}C$) in 80 individuals revealed no significant differences in location or sex, and the species was classified as secondary consumer with a trophic level (TL) of 2.5. This study provides valuable insights into the population dynamics of *R. venosa* and its ecological impact on the local and regional ecosystems.

**Key words:** Biological invasion, population dynamics, ecological role, isotopic signals, trophic level, ecosystem impact.

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**RESUMEN.** *Rapana venosa* es un gasterópodo de origen asiático e invasor exitoso en ecosistemas costero-estuarinos del mundo. Registrada para el Río de la Plata (RdIP) en 1999, se extiende actualmente hacia el sur por la costa atlántica argentina, y al norte por la costa atlántica uruguaya y sur de Brasil. Se obtuvieron muestras de cruceros de evaluación pesquera durante la primavera del 2017 en el RdIP (medio/externo) y en la costa uruguaya de la Zona Común de Pesca Argentino-Uruguay. En 16% de las estaciones se capturaron ejemplares con red de arrastre de fondo y una densidad de 3.88 kg m⁻². En los 119 ejemplares analizados, los machos fueron dominantes y los dos organismos se identificaron como imposto. La mayoría de organismos presentó una alta cobertura de epibiontes y 10% se encontraron consumiendo bivalvos autóctonos. Las señales isotópicas ($\delta^{15}N$ y $\delta^{13}C$) en los 80 individuos no variaron significativamente entre sitios o sexos, y el nivel trófico (NT = 2,5) cataloga a la especie como consumidor secundario. Este estudio proporciona información sobre la dinámica...
Introduction

Rapana venosa (Valenciennes, 1846) is a benthic gastropod native to eastern Asian seas, including the Sea of Japan, the Yellow Sea, and the East China Sea. Its successful invasive behavior in various coastal and estuarine ecosystems worldwide can be attributed to its high tolerance to salinity and temperature variations, as well as its adaptation to low oxygen concentrations (ICES 2004). The first record of this species outside its native range was in the Black Sea in 1946 (Drapkin 1953). Since then, established populations have been identified in the Adriatic and Aegean Seas, coasts of Brittany, the Netherlands, Chesapeake Bay, Río de la Plata, and recently was reported in the southern coast of Brazil (ICES 2004; Sportono-Oliveira et al. 2020). In the late 1990s, it was first documented in the estuary of the Río de la Plata (RdIP) on the Uruguayan coast (Maldonado Bay) (Scarabino et al. 1999) and the Argentine coast (Samborombón Bay) (Pastorino et al. 2000). Currently, it is expanding both southward along the Atlantic coast of Argentina (Giberto and Bruno 2014) and northward along the coast of Uruguay (Laporta et al. 2018 Sep 14).

Rapana venosa is widely distributed in muddy bottoms of the subtidal myxohaline zone of the RdIP, as well as in rocky shores and mussel beds (Giberto et al. 2006; Lanfranconi et al. 2009; Carranza et al. 2010; Brugnoli et al. 2014; Giberto and Bruno 2014). Its colonization success is attributed to the availability of bivalve mollusks as a food source, and the lack of direct predators and potential competitors in coastal areas of Uruguay and Argentina (Giberto et al. 2006; Lanfranconi et al. 2013). Bruno (2016) highlights that this successful colonization of the RdIP was due to the combination of species characteristics such as being an r and k strategist, including generalist habits, rapid sexual maturity (one year), a high number of offspring, large body size, and long life expectancy (> 15 years), combined with a successful incorporation into estuarine trophic webs. This species presents both infaunal and epifaunal behavior, inhabiting intertidal and subtidal areas of sandy and rocky mud, being an active predator that poses a serious threat to the diversity of native mollusks and the structure of the ecosystem (Giberto et al. 2006; Lanfranconi et al. 2013). Previous studies on this invasive species in the RdIP have focused mainly on the analysis of impacts, distribution, predation rates on native mollusks, biofouling effects on sea turtles, predators (Caretta, Mustelus), description of the invasion progress, and genetic diversity at different invasion sites globally, including the RdIP (Carranza et al. 2008; Lanfranconi et al. 2009; Giberto et al. 2011; Lezama et al. 2012; Giberto and Bruno 2014; Muniz et al. 2021; Xue et al. 2018).

Population studies are crucial to understand population size, sex ratios, feeding behavior, and habitat use. Additionally, analyzing isotopic signals of Nitrogen and Carbon ($\delta^{15}$N and $\delta^{13}$C) simultaneously allows determining the source of organic matter consumed by organisms and estimating their trophic position in the food web (Michener and Kaufman 2007). This information helps comprehend the dynamics and structure of biological communities. The first characterization of the isotopic signal of R. venosa in the RdIP was conducted by Botto et al. (2011), placing it as a consumer link in the estuarine trophic web. The present study aimed to assess the presence, abundance, and various population parameters of R. venosa in the Río de la Plata (RdIP) during spring 2017 and update their invasion status. These parameters include size, weight, sex ratios, feeding and ecological behavior,
isotopic signals of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ (analyzed with consideration for site, sex, and length), and population trophic level (TL).

**MATERIAL AND METHODS**

**Study area**

The Río de la Plata is a funnel-shaped estuary located between $34^\circ$ S-$36^\circ$ S and $55^\circ$ W-$58^\circ$ W on the east coast of South America. It drains the second-largest basin on the continent into the Atlantic Ocean, covering 3.1 million km² (Acha et al. 2008). This fluvial-estuarine-maritime system is 290 km long, has a maximum width of 220 km at its mouth, and is relatively shallow (depth up to 29 m) with a high river flow and gentle slope. It is a microtidal system with subtidal oscillations similar in amplitude to astronomical tidal oscillations. The tidal amplitude on the Argentine coast is 1 m, while on the Uruguayan coast, it is 0.4 m. Mean annual river discharge to the Río de la Plata is approximately 25,000 m$^3$ s$^{-1}$, with the Paraná and Uruguay rivers as the main tributaries. This discharge oscillates depending on dry/wet periods associated with La Niña/El Niño events (Nagy et al. 2002, 2008, 2023).

It is estimated that the input of sediment to the Río de la Plata is approximately 79.8 billion kg year$^{-1}$ (Framiñan et al. 1999). This sediment consists primarily of suspended silts and clays, comprising around 90% of the total sediment load, with the remaining 10% consisting of very fine sands (Pittau 2007). Suspended sediment concentrations in the Río de la Plata exhibit a range from 60 to 300 mg l$^{-1}$, with higher concentrations often associated with resuspension during storm events (Fossati et al. 2014). Extreme concentrations of nearly 400 mg l$^{-1}$ have been reported in certain instances (Moreira et al. 2013). Bottom sediments exhibit gradual settlement along the estuary. Towards the head of the estuary, sand predominates, whereas in the middle estuary, silt is the dominant sediment type. As one moves towards the mouth of the estuary, clay-silt and clay become increasingly prevalent. Notably, around Barra del Indio, clay predominates, and this region is characterized by elevated water and organic matter content, with physical-chemical flocculation processes playing a significant role. In the outer region, closer to the seaward edge and the salt wedge, coarse sand becomes the dominant sediment type (Moreira et al. 2016).

The bathymetry and geomorphology of the Río de la Plata bed exhibit significant variation and can be categorized into upper (2,700 km$^2$), intermediate (9,000 km$^2$), and outer (25,000 km$^2$) regions. The upper region is a shallow shelf, with depths ranging from approximately 1 to 4 m and characterized by a gentle slope. Here, the main channel, formed by the confluence of the Paraná and Uruguay rivers, follows the Uruguayan coast and leads to the Ortiz Grande bank. This bank, with a maximum depth of nearly 3 m, plays a key part in bifurcating the channel. The intermediate region features depths of less than 10 m and is home to the Intermediate channel, situated along the southern shore. In the outer region, depths range from 10 to 20 m, with the Eastern channel found on the north coast and the Maritime channel on the south coast. Depths exceeding 20 m are observed after Punta del Este-Punta Rasa (Venturini et al. 2023 and references herein).

**Sample collection**

During spring 2017, as part of coastal fishery assessment cruises conducted out by the National Directorate of Aquatic Resources of the Ministry of Agriculture, Livestock and Fishery (DINARA-MGAP, Uruguay) onboard the ‘Aldebarán’ research vessel, a total of 51 fishing hauls were carried out. These hauls spanned depths ranging from 6 to 70 m within the Argentine-Uruguayan Common Fishing Zone between $34^\circ$ S-$36^\circ$ S (Figure 1). Benthic fauna samples were collected using an Engel bottom trawl equipped with a minimum mesh size of 35 mm between knots. The presence or absence of *R. venosa* was documented at differ-
ent stations, and in instances where the species was present, the captured organisms were quantified and subsequently refrigerated for further analysis at the Oceanography and Marine Ecology laboratories (School of Sciences, Montevideo).

**Laboratory analysis**

In stations where *R. venosa* was observed, a random subsampling method was employed, selecting 119 organisms for the determination of various morphological parameters, identification, and quantification of epibionts. Morphological traits, including total length and width (mm) of the shells, were measured with a manual caliper graduated to 0.1 mm. The total weight of each organism (comprising both shell weight and soft part weight) was determined using an electronic balance with a 0.01 g accuracy. Sexual characteristics, including sex (male, female, or indeterminate) and the presence of imposex, were determined following the methodology outlined by Besozzi (2013). The coverage of epibionts on the shells was recorded, and epibionts were identified.

Among sampled individuals, soft parts (muscular foot) from 80 organisms were extracted for stable isotope analysis ($\delta^{15}N$ and $\delta^{13}C$). These soft parts were dried for 48 h at 60 °C, macerated into a fine powder, weighed to 1.00 mg using a precision scale with a resolution of 0.0001 mg, and then placed in tin capsules for shipment. The isotopic analysis was conducted using an Elemental Analyzer Continuous Flow Isotope Ratio Mass Spectrometry setup at the Center for Stable Isotopes, University of New Mexico. The system involved a Costech ECS 4010 Elemental Analyzer connected to a Thermo Fisher Scientific Delta V Advantage mass spectrometer through a CONFLO IV interface.

**Data analysis**

A distribution map depicting organism densities (in weight: kg mn$^{-2}$) across the study area was generated. Isotopic signals were calculated based on
δ¹⁵N and δ¹³C values, following the formula: δ¹⁵N and δ¹³C = [(Rsample/Rstandard) - 1] × 1,000 (‰), where R represents the ¹³C/¹²C or ¹⁵N/¹⁴N ratio for δ¹³C and δ¹⁵N, respectively.

Relationships between morphometric parameters and isotopic signals (δ¹⁵N and δ¹³C) were explored, and the trophic level (TL) of the *R. venosa* species was determined using the formula: TL = (δ¹³N Rapana - δ¹³N Mactra) / 3.4 + 2 (Botto et al. 2011).

Non-parametric statistical analyses (Kruskal-Wallis) were performed at a significance level of 95% to evaluate differences in length and weight between males and females, as well as differences in isotopic signals according to sex or seasons.

**RESULTS**

*Rapana venosa* displayed a widespread distribution within the myxohaline zone of the RdIP, occurring between the 5 m and 20 m isobaths. Specimens were captured in 16% of the hauls made (Figure 1). Densities of *R. venosa* exhibited a wide range, spanning from 3.85 to 10,764 kg m⁻². Notably, there was a discernible decrease in densities with increasing distance from the coastline, ranging from 0-100 kg m⁻². The highest densities were observed in the region between departments of Montevideo and Maldonado, where they reached levels of 101-1,000 kg m⁻² and 5,001-11,000 kg m⁻², respectively (Figure 2).

Among 119 individuals analyzed, males were predominant (54%); however, it should be noticed that this trend varied across different sampling stations. A total of 65 males and 52 females were counted (Figure 3 A and 3 B), while imposex was observed in two individuals (Figure 3 C). The observed size of these organisms ranged from 51.5 mm to 137.6 mm, with a mean shell length of 97.6 ± 13.8 mm and a mean shell width of 71.2 ± 11.1 mm (ranging from 36.6 to 97.1 mm) (Figure 4). The total weight ranged from 28.34 g to 373.55 g, with soft parts accounting for 30% of the total weight and the remaining 70% attributed to
the shell. No significant differences were detected between males and females in terms of both total length ($p = 0.82$) and total weight ($p = 0.63$).

A noteworthy 95% of analyzed individuals exhibited a high number of epibionts on the external surface of their shells, with coverage exceeding 40% (Figure 5 A and 5 B). The most prevalent epibiont organisms identified were bryozoans (Membranipora sp.) (87%) and barnacles (Balanus sp.) (74%). Coelenterates (Anemone sp.), annelids (Polydora sp.), and mollusks (Ostrea sp.) were also present but in smaller quantities (< 10%). Furthermore, 14% of individuals showed an ovigerous mass on their shells (Figure 5 C). Additionally, 10% of the organisms were identified with Mactra isabelleana, inside the shells (Figure 5 D).

Average isotopic signals of $R. \textit{venosa}$ were determined to be $\delta^{15}N: 13.1 \pm 0.7$ and $\delta^{13}C: -15.9 \pm 0.5$ (Figure 6 A-C). Notably, there were no significant differences in isotopic signals between sampling stations ($p \delta^{13}C = 0.16, p \delta^{15}N = 0.31$) or between sexes (p-value = 0.46). Regarding total length (mm), there was greater variation in $\delta^{15}N$, although no clear trend emerged (Figure 6 D). The calculated trophic level was determined to be 2.5, classifying the species as a secondary consumer.

**DISCUSSION**

The distribution pattern of $R. \textit{venosa}$ within the myxohaline zone of the Río de la Plata is in line with previous studies, indicating its wide presence between depths of 4 to 26 m (Giberto et al. 2006; Carranza et al. 2008). Since its initial record in the RdLP (Scarabino et al. 1999; Pastorino et al. 2000), this species has effectively expanded along the Atlantic coast, extending its range into eastern Uruguay, southern Brazil, and southern Argentina (Giberto and Bruno 2014; Laporta et al. 2018; Spotorno-Oliveira et al. 2020; Muniz et al. 2021). However, this expansion does not align with the assertion made by Lanfranconi et al. (2009), suggesting that the species might encounter its distribution limit in the Punta del Este area due to competition with the native gastropod Stramonita haemastoma. Despite the expansion of its distribution range in littoral and coastal areas of the RdLP, the sampling stations (5-20 m) with presence of organism corre-
spond to the distribution area previously reported by other authors (Carranza et al. 2008, 2010; Giberto and Bruno 2014).

Densities of *R. venosa* reported in this study were lower compared to those found in the North Sea (Saglam et al. 2015; Gönener and Özsandıklı 2017). Notably, these studies focused on the determination of densities and the assessment of fish stocks for exploitation using trawls with different dimensions than those used in this study. In contrast, our study collected *R. venosa* specimens during fishing research surveys, where this species was part of the accompanying fauna. Future studies that focus specifically on the evaluation of *R. venosa* in the RdLP could provide a more accurate estimate of the magnitude of its invasion and enable comparisons with other invaded systems.

The reported size range of *R. venosa* individuals aligns with previous research in the estuarine area (Giberto et al. 2006) and in other invaded coastal ecosystems globally (Savini et al. 2004; Saglam et al. 2015). The absence of juveniles in our study is consistent with previous findings and could be attributed to factors such as gear selectivity, predation, or differences in habitat preferences compared to adults (Harding and Mann 1999; Savini et al. 2004). In our case, the size of the net used (with a mesh size of 35 mm between knots) may have in-

Figure 4. Population characteristics of *Rapana venosa* in the study area (n = 119 individuals), including the proportion of males and females (A), average total weight (B), average total length (C), and average total width (D).
fluenced the results, biasing the collection towards predominantly adult organisms.

The number of individuals presenting epibiont coverage on their shells, as well as the most common epibiont organisms (bryozoans and barnacles) agrees with previous work conducted in the RdIP estuary (Giberto et al. 2006), and are also similar to those reported by Savini et al. (2004). Giberto et al. (2006) considered organisms from the RdIP in similar habitats (sedimentary environments, or unconsolidated substrates), while Savini et al. (2004) analyzed individuals from consolidated substrates (rock). Although some authors mention that the infaunal behavior of *R. venosa* would prevent the settlement of epibiota on its shells (Harding and Mann 1999, 2005), results found for the RdIP suggest that in this region the species presents a mostly exposed lifestyle, favored by conditions of low light incidence and high estuarine sedimentation (Giberto et al. 2006). Furthermore, the presence of egg-masses of this species on its shell highlights its role as a substrate for the settlement of larvae of organisms not typically found on muddy estuarine bottoms (Giberto et al. 2004, 2006).

Imposex is a phenomenon characterized by the development of male sexual characteristics (such as a penis and/or *vas deferens*) in female gastropods. This condition arises due to exposure to or-
ganic tin contaminants, with tri-butyl-tin (TBT) being a notable example, primarily found in antifouling paints (Bigatti et al. 2014). This phenomenon has been extensively documented in coastal areas around the world since 1969. In South America, there have been reports of imposex in 33 species of gastropods (Bigatti et al. 2014). In the case of the Río de la Plata region, there is prior evidence of imposex occurring in *R. venosa* (Besozzi 2013). In our current study, 1.7% prevalence of imposex was identified in organisms examined, marking the first report of this phenomenon in the studied area. It is important to note that the number of specimens exhibiting imposex was considerably lower than what Besozzi (2013) reported in collections conducted in port areas of the RdIP. This difference may be attributed to variations in environments where individuals were collected. In our study, specimens were gathered from the estuarine subtidal zone of the RdIP, which is notably distant from port areas. In contrast, Besozzi (2013) worked with specimens obtained from two port areas, namely Piriápolis and Punta del Este. Harbor areas are more likely to have vessels, including small and tourist boats like yachts that use TBT-containing antifouling paints. This exposure to such paints could explain the higher imposex prevalence observed in Besozzi’s collections.

Figure 6. Isotopic signals of *Rapana venosa* in the Río de la Plata, including sampling stations (A), males and females (B), and variations in total length versus δ¹³C and δ¹⁵N (C and D).
Isotopic signals of *R. venosa* from this study closely resemble previous findings by Botto et al. (2011) in the RdIP. However, $\delta^{13}C$ values in our study were notably lower than those reported by Botto et al. (2011). This discrepancy may stem from the larger number of specimens ($n = 80$) used in our analysis, which holds significance for future trophic studies in the RdIP. Moreover, the consistency of isotopic signals across different seasons, sexes, and sizes, without statistically significant differences, may suggest a consistent dietary supply and environmental homogeneity for this species in the study area. Nevertheless, comprehensive future studies should encompass a wider size range and a broader temporal scope to address these questions definitively.

The determined trophic level (TL $= 2.5$) classifies the species as a secondary consumer, in accordance with Botto et al. (2011). This classification aligns with prior research that identified *R. venosa* as a predator of native mollusks and other invertebrates in the RdIP (e.g. Giberto et al. 2006, 2011; Lanfranconi et al. 2009; Carranza et al. 2010). The coexistence of *R. venosa* with native bivalve mollusk species such as *Mactra isabelleana* and *Ostrea puelchana* suggests potential predation effects on these native species (Giberto et al. 2006; Brugnoli et al. 2014). In our study, 10% of organisms were found consuming bivalves like *Mactra isabelleana*, indicating active predation on native mollusk species. Additionally, predatory behavior towards native *Mytilus* spp. and *Brachidontes* spp. mussels has been observed through visual surveys and controlled laboratory experiments (Carranza et al. 2010; Giberto et al. 2011; Lanfranconi et al. 2013). However, the long-term ecological and socioeconomic impacts of *R. venosa* on native mollusks in the RdIP have yet to be quantified (Brugnoli et al. 2014; Carranza et al. 2023). According to trophic models developed for the RdIP, *R. venosa* is considered a key species in the trophic dynamics of the estuary (Lercari and Bergamino 2011). Negative effects of this snail extend beyond predation and may include competition with other native predators, such as the gastropod *Stramonita haemastoma*, which *R. venosa* might displace or reduce in numbers (Lanfranconi et al. 2009; Muniz et al. 2021).

In this context, as reported by Lanfranconi et al. (2009), the distribution limit of *R. venosa* along the coast of Uruguay during the time of their study, was primarily constrained to Punta del Este. This limitation was mainly attributed to competition with native species. However, a more recent study by Laporta et al. (2018) reveals that the distribution of *R. venosa* has since expanded eastward along the Uruguayan coast. Currently, both *R. venosa* and native species share overlapping distributions, with the potential for unknown impacts, such as competition, between them. Additionally, the presence of *R. venosa* directly affects the green turtle (*Chelonia mydas*). These snails adhere to the turtle’s shell, impairing its buoyancy and hindering its swimming ability, sometimes causing it to sink (Lezama et al. 2012).

Like all invasive species, *R. venosa* presents the potential to generate socio-environmental, biodiversity, and ecosystem impacts. Once an invasive population establishes itself, eradication becomes nearly impossible (Thresher and Kuris 2004). This challenge is particularly prominent in marine-coastal ecosystems, where recommended measures include surveillance, entry prevention, and early detection of potentially invasive marine organisms (Lehtiniemi et al. 2015; Sankaran et al. 2023). In Uruguay, the management of invasive species has been marred by biases, primarily stemming from the lack of an ecosystem-oriented approach and the absence of surveillance, prevention, or immediate population control measures once an invasion begins (Brazeiro et al. 2021). In the case of *R. venosa*, some population control measures and strategies have been implemented, including the use of traps (pots) in the RdIP (Argentina) (Schiariti et al. 2011) and manual extraction by artisanal mussel farmers in Uruguay (Carranza et al. 2010; Muniz et al. 2021). Furthermore, outreach and awareness campaigns incentivized the population to remove these organisms, offering
remuneration for each one collected (Brugnoli et al. 2014). However, these efforts have been temporarily discontinued and are currently on a reduced scale, hindering long-term population control that could alleviate the environmental impacts of this species.

CONCLUSIONS

This study presents density records, morphometric parameters, and isotopic signal values ($\delta^{15}$N and $\delta^{13}$C) for a high number of $R.$ venosa individuals in the RdlP. These data provide essential insights for future research aimed at better understanding the population dynamics of this invasive alien species and its ecological repercussions. Future investigations should encompass a broader range of size categories and a more extensive temporal and spatial scope. A multidisciplinary approach is imperative to understand the complexity and magnitude of the long-term consequences of this invasive alien species on local ecosystems and neighboring regions, with the ultimate goal of preventing its further expansion.

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Author contributions

Maite Sánchez Acosta: methodology, formal analysis, data curation, writing-original draft, writing-review and editing. Noemí Góngora: methodology, formal analysis, data curation, writing-original draft, writing-review and editing. Diego Antuña: methodology, data curation, formal analysis. Patricia Correa: resources, writing-review and editing. Ernesto Chiesa: formal analysis, resources, data curation, writing-review and editing. Ernesto Brugnoli: conceptualization, resources, supervision, project administration, funding acquisition, writing-original draft, writing-review and editing. Pablo Muniz: conceptualization, resources, supervision, project administration, funding acquisition, writing-original draft, writing-review and editing.

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